

C1  
1. A single [cDNA] ~~DNA~~ comprising a nucleic acid sequence coding for cystic fibrosis transmembrane conductance regulator.

Sub D1  
Please add the following claims:

25 2/ The DNA of claim 1 that is ~~stabilized~~ against cellular recombination.

26 3/ The DNA of claim 1 further comprising at least one intron located within the cystic fibrosis transmembrane conductance regulator coding region.

Sub D2  
27 4/ The DNA of claim 3/ wherein the DNA has the intervening sequence set forth in Figure 6.

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28 5/ The DNA of claim 1 contained within a vector having regulatory elements for enabling transcription of the DNA.

29 28  
6/ The DNA of claim 5/ wherein the vector is pSC-CFTR2.

30 7/ The DNA of claim 1 further comprising phage, viral, liposome or virosome elements for enabling introduction of the DNA encoding cystic fibrosis transmembrane conductance regulator into a cell.

31 30  
8/ The DNA of claim 7/ wherein the phage or retroviral elements comprise elements selected from the group consisting of bacteria phage lambda, retroviruses or vaccinia viruses.

<sup>32</sup>  
~~9.~~ The DNA of claim 1 wherein cryptic regulatory sequences have been removed without altering the cystic fibrosis transmembrane conductance regulator.

<sup>33</sup>  
~~10.~~ The DNA of claim <sup>36</sup>~~7~~ formulated into a therapeutically effective composition.

<sup>34</sup>  
~~11.~~ The DNA of claim 1 comprising the amino acid encoding sequence set forth in Table 1.

<sup>35</sup>  
~~12.~~ The DNA of claim <sup>34</sup>~~11~~ further comprising at least one element for stabilizing the DNA.

<sup>36</sup>  
~~13.~~ A low copy number vector comprising DNA encoding cystic fibrosis transmembrane conductance regulator.

<sup>37</sup>  
~~14.~~ The vector of claim <sup>36</sup>~~13~~ wherein the DNA is cDNA.

<sup>38</sup>  
~~15.~~ The vector of claim <sup>36</sup>~~13~~ wherein the DNA further comprises at least one intron located within the amino acid encoding region of cystic fibrosis transmembrane conductance regulator.

<sup>39</sup>  
~~16.~~ The vector of claim <sup>38</sup>~~15~~ comprising the vector pkk-CFTR3.

<sup>40</sup>  
~~17.~~ The vector of claim <sup>36</sup>~~13~~ further comprising a stabilizing element.

<sup>41</sup>  
~~18.~~ The vector of claim <sup>36</sup>~~13~~ wherein the DNA codes for cystic fibrosis transmembrane conductance regulator and comprises a point mutation or deletion found in patients suffering from cystic fibrosis.

- 42/ 19. The vector of claim 18<sup>41</sup> which comprises at least one point mutation or deletion within the coding region for cystic fibrosis transmembrane conductance regulator.
- 43/ 20. The vector of claim 19<sup>42</sup> wherein the cystic fibrosis transmembrane conductance regulator encoded by said DNA lacks a phenylalanine at amino acid position 508.
- 44/ 21. The vector of claim 13<sup>36</sup> which, when introduced into a human, is capable of producing cystic fibrosis transmembrane conductance regulator.
- 45/ 22. The vector of claim 13<sup>36</sup> wherein the copy number in *E. coli* is less than about 25 per cell.
- 46/ 23. The vector of claim 13<sup>36</sup> wherein the vector is present in a copy number which, when the vector is introduced into a host cell, does not result in the production of cystic fibrosis transmembrane conductance regulator in a quantity or concentration which causes the host cell to die.
- 47/ 24. A single DNA comprising a nucleic acid sequence coding for a molecule having the biological activity of cystic fibrosis transmembrane conductance regulator.
- 48/ 25. An isolated DNA sequence encoding a protein having cystic fibrosis transmembrane conductance regulator biological activity.
- 49/ 26. The sequence of claim 25<sup>48</sup> which is a cDNA.
- 50/ 27. A purified DNA comprising the amino acid encoding sequence shown in Figure 6.

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- 51  
28. A vector comprising the DNA sequence of claim 27. 50
- 52  
29. A phage, viral, liposome or virosome comprising the DNA sequence of claim 27. 50
- 53  
30. A therapeutic composition comprising the phage, virus, liposome or virosome of claim 29 capable of effecting the production of cystic fibrosis transmembrane conductance regulator in humans. 52
- 54  
31. A therapeutic composition comprising a carrier comprising the vector of claim 28 which after administration, augments the *in vivo* production or activity of cystic fibrosis transmembrane conductance regulator. 51
- 55  
32. A host cell comprising the DNA of claim 1, the DNA of claim 13, the DNA of claim 24, the DNA of claim 28, or the DNA of claim 21. 36 47 50
- 56  
33. The host cell of claim 32 wherein the host cell is a eucaryotic cell. 48 55
- 57  
34. The host cell of claim 33 wherein the host cell is a mammalian cell. 56
- 58  
35. The host cell of claim 34 wherein the host cell is selected from the group consisting of C127, myeloma and CHO cells. 57
- 59  
36. The host cell of claim 33 selected from the group consisting of insect cells, fungi and plant cells. 56

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<sup>60</sup>  
~~37.~~ The host cell of claim ~~32~~<sup>55</sup> wherein the host cell is a prokaryotic cell.

<sup>61</sup>  
~~38.~~ The host cell of claim ~~37~~<sup>60</sup> comprising *E. coli*.

<sup>62</sup>  
~~39.~~ A host cell comprising the ~~DNA~~<sup>25</sup> of claim ~~2~~<sup>26</sup>, the DNA of claim ~~3~~<sup>38</sup>, the vector of claim ~~18~~<sup>42</sup> or the vector of claim ~~19~~<sup>42</sup>

<sup>63</sup>  
~~40.~~ The host cell of claim ~~39~~<sup>62</sup> wherein the host cell is a eucaryotic cell.

<sup>64</sup>  
~~41.~~ The host cell of claim ~~40~~<sup>63</sup> wherein the host cell is a mammalian cell.

<sup>65</sup>  
~~42.~~ The host cell of claim ~~41~~<sup>64</sup> wherein the host cell is selected from the group consisting of C127, myeloma and CHO cells.

<sup>66</sup>  
~~43.~~ The host cell of claim ~~39~~<sup>62</sup> selected from the group consisting of insect cells, fungi and plant cells.

<sup>67</sup>  
~~44.~~ The host cell of claim ~~39~~<sup>62</sup> wherein the host cell is a prokaryotic cell.

<sup>68</sup>  
~~45.~~ The host cell of claim ~~44~~<sup>67</sup> comprising *E. coli*.

<sup>69</sup>  
~~46.~~ Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim ~~32~~<sup>55</sup>.

70  
47. Cystic fibrosis transmembrane conductance regulator isolated from a mammalian host cell comprising purified DNA or single DNA sequence encoding a protein having cystic fibrosis transmembrane conductance regulator activity.

71  
48. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 37.60

72  
49. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 38.61

73  
50. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 39.62

74  
51. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 41.64

75  
52. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 42.65

76  
53. Cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 44.67

77  
54. A therapeutically effective composition for treating cystic fibrosis comprising the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 32. 53

- 78  
55. A therapeutically effective composition for treating cystic fibrosis comprising the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 34. 57
- 79  
56. A therapeutically effective composition for treating cystic fibrosis comprising the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 39. 62
- 80  
57. A therapeutically effective composition for treating cystic fibrosis comprising the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 41. 64
- 81  
58. The therapeutically effective composition of claim 54 further comprising a carrier for delivering the composition to cells requiring augmentation of cystic fibrosis transmembrane conductance regulator function. 77
- 82  
59. The therapeutically effective composition of claim 55 further comprising a carrier for delivering the composition to cells requiring augmentation of cystic fibrosis transmembrane conductance regulator function. 78
- 83  
60. The therapeutically effective composition of claim 56 further comprising a carrier for delivering the composition to cells requiring augmentation of cystic fibrosis transmembrane conductance regulator function. 79
- 84  
61. The therapeutically effective composition of claim 57 further comprising a carrier for delivering the composition to cells requiring augmentation of cystic fibrosis transmembrane conductance regulator function. 80

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<sup>85</sup>  
~~62.~~ The therapeutically effective composition of claim ~~54~~<sup>77</sup> which is introduced intranasally or by respiratory aerosol.

<sup>86</sup>  
~~63.~~ The therapeutically effective composition of claim ~~56~~<sup>79</sup> which is introduced intranasally or by respiratory aerosol.

<sup>87</sup>  
~~64.~~ A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the DNA of claim 1, the DNA of claim ~~3~~<sup>26</sup> or the DNA of claim ~~7~~<sup>30</sup>.

<sup>88</sup>  
~~65.~~ A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the vector of claim ~~13~~<sup>36</sup> or the vector of claim ~~21~~<sup>44</sup>.

<sup>89</sup>  
~~66.~~ A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the DNA of claim ~~25~~<sup>40</sup>.

<sup>90</sup>  
~~67.~~ A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the phage of claim ~~29~~<sup>52</sup>.



- 91 68. A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 34. 57
- 92 69. A method for treating a disease condition having the characteristics of cystic fibrosis comprising the step of administering to cells having defective cystic fibrosis transmembrane conductance regulator function a therapeutically effective dose of the cystic fibrosis transmembrane conductance regulator isolated from the host cell of claim 39. 62
- 93 70. The method of claim 69 wherein the host cells are mammalian cells. 92
- 94 71. A method for screening compositions for selecting therefrom compounds capable of affecting cystic fibrosis transmembrane conductance regulator function comprising the steps of contacting said compositions to be screened with the host cell of claim 32 and detecting those compounds which affect the cystic fibrosis transmembrane conductance regulator phenotype of said cell. 55
- 95 72. The method of claim 71 wherein said compounds are selected on the basis of their binding to the cystic fibrosis transmembrane conductance regulator of said cell. 94
- 96 73. A method for screening compositions for selecting compounds capable of affecting cystic fibrosis transmembrane conductance regulator function comprising the steps of contacting said compositions to be screened with the host cell of claim 39 and detecting those compounds which affect the cystic fibrosis transmembrane conductance regulator phenotype of said cell. 62

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- 97  
74. A method for assaying for cystic fibrosis transmembrane conductance regulator function comprising the improvement of using as a positive control, cystic fibrosis transmembrane conductance regulator produced by the host cell of claim 32. 55
- 98  
75. A method for assaying for cystic fibrosis transmembrane conductance regulator function comprising the improvement of using as a positive control, cystic fibrosis transmembrane conductance regulator produced by the host cell of claim 39. 62
- 99  
76. A method for producing cystic fibrosis transmembrane conductance regulator comprising the steps of culturing the host cells of claim 32 under conditions permitting expression of the cystic fibrosis transmembrane conductance regulator and isolating from said cells, said cystic fibrosis transmembrane conductance regulator. 55
- 100  
77. A method for producing cystic fibrosis transmembrane conductance regulator comprising the steps of culturing the host cells of claim 39 under conditions permitting expression of the cystic fibrosis transmembrane conductance regulator and isolating from said cells, said cystic fibrosis transmembrane conductance regulator. 62
- 101  
78. A kit for assessing cystic fibrosis transmembrane conductance regulator function comprising the host cells of claim 32 packaged in a container, and instructions. 55
- 102  
79. A kit for assessing cystic fibrosis transmembrane conductance regulator function comprising the host cells of claim 39 packaged in a container, and instructions. 62

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- 103/ 80. A kit comprising cystic fibrosis transmembrane conductance regulator agent means produced by the host cells of claim <sup>55</sup>32 packaged in a container, and instructions.
- 104/ 81. The kit of claim <sup>103</sup>80 wherein said cystic fibrosis transmembrane conductance regulator agent means comprises cystic fibrosis transmembrane conductance regulator or cell membranes containing said cystic fibrosis transmembrane conductance regulator.
- 105/ 82. A kit comprising cystic fibrosis transmembrane conductance regulator agent means produced by the host cells of claim <sup>60</sup>39 packaged in a container, and instructions.
- 106/ 83. The therapeutically effective composition of claim <sup>17</sup>54 which is introduced intranasally or by respiratory aerosol.
- 107/ 84. The therapeutically effective composition of claim <sup>79</sup>56 which is introduced intranasally or by respiratory aerosol.
- c2  
cont. 108/ 85. A transgenic animal comprising the DNA of claim 1, the DNA of claim <sup>36</sup>13, the DNA of claim <sup>47</sup>24, the DNA of claim <sup>40</sup>25, or the DNA of claim <sup>30</sup>27.
- 109/ 86. The cystic fibrosis transmembrane conductance regulator collected from the transgenic animal of claim <sup>108</sup>85.
- 110/ 87. A transgenic animal comprising the DNA of claim <sup>25</sup>2, the DNA of claim <sup>26</sup>3, the vector of claim <sup>36</sup>15 or the vector of claim <sup>42</sup>19.

- <sup>111</sup> 88. The cystic fibrosis transmembrane conductance regulator collected from the transgenic animal of claim <sup>110</sup> 87.
- <sup>112</sup> 89. A recombinant cystic fibrosis transmembrane conductance regulator protein.
- <sup>113</sup> 90. A recombinant cystic fibrosis transmembrane conductance regulator protein produced from a host cell comprising a single DNA sequence.
- <sup>114</sup> 91. The recombinant cystic fibrosis transmembrane conductance regulator protein of Claim <sup>113</sup> 90, wherein the single DNA sequence is as shown in Table 1.
- <sup>115</sup> 92. The recombinant cystic fibrosis transmembrane conductance regulator protein of Claim <sup>114</sup> 91 wherein the single DNA sequence contains a synthetic intron between nucleotide positions 1716 and 1717.
- <sup>116</sup> 93. The recombinant cystic fibrosis transmembrane conductance regulator protein of Claim <sup>113</sup> 90 wherein the single DNA sequence is contained in a low copy number vector.
- <sup>117</sup> 94. A recombinant cystic fibrosis transmembrane conductance regulator protein having an amino acid sequence as shown in Table 1, said protein being produced from a host cell comprising a single DNA sequence having a mutation in a cryptic regulatory sequence.
- <sup>118</sup> 95. A therapeutic composition for treating cystic fibrosis comprising a therapeutically effective amount of the cystic fibrosis transmembrane conductance regulator protein of Claim <sup>112</sup> 89 and a therapeutically acceptable carrier.

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- 119  
96. A therapeutic composition for treating cystic fibrosis comprising therapeutically effective amount of the cystic fibrosis transmembrane conductance regulator protein of Claim <sup>113</sup>90 and a therapeutically acceptable carrier.
- 120  
97. A therapeutic composition for treating cystic fibrosis comprising a therapeutically effective amount of the cystic fibrosis transmembrane conductance regulator protein of Claim <sup>114</sup>91 and a therapeutically acceptable carrier.
- 121  
98. A therapeutic composition for treating cystic fibrosis comprising a therapeutically effective amount of the cystic fibrosis transmembrane conductance regulator protein of Claim <sup>116</sup>93 and a therapeutically acceptable carrier.
- 122  
99. An antibody specific for an epitope of the cystic fibrosis transmembrane conductance regulator.
- 123  
100. The antibody of Claim <sup>122</sup>99 which is a monoclonal antibody.
- 124  
101. The monoclonal antibody of Claim <sup>123</sup>100 having at least one of the identifying immunological characteristics of antibodies produced by cells ATCC Accession No. HB 10565 or ATCC Accession No. HB 10566.
- 125  
102. A method for detecting the presence of the cystic fibrosis transmembrane conductance regulator in a biological sample comprising the steps of:

- 123
- a) contacting said biological sample with the monoclonal antibody of Claim 100 under conditions conducive to permit immunological complexes to form,
- b) allowing the monoclonal antibody to bind to the cystic fibrosis transmembrane conductance regulator to form an immunological complex, and
- c) detecting the formation of said immunological complex and correlating the presence or absence of said immunological complex with the presence or absence of cystic fibrosis transmembrane conductance regulator in the biological sample.

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103. A method for obtaining purified cystic fibrosis transmembrane conductance regulator from an impure solution containing said regulator comprising the steps of:

- 123
- a) contacting said impure solution with the monoclonal antibody of Claim 100,
- b) allowing the monoclonal antibody to bind to said regulator to form a complex, and
- c) separating the complex from the impure solution.

127  
104. The method of Claim 103 wherein the monoclonal antibody is immobilized on a resin support.

128  
105. The method of Claim 104 wherein the separation is carried out using immunoaffinity chromatography.

- <sup>129</sup>106. The antibody of Claim <sup>122</sup>99 wherein the epitope is selected from the group consisting of Exon 13, Exon 1, Exon 10, Exon 24 and extracellular loops approximately defined by amino acids 881-911.
- <sup>130</sup>107. The antibody of Claim <sup>123</sup>100 which is an Fab or an F(ab')<sub>2</sub>.
- <sup>131</sup>108. A method for producing antibodies specific for cystic fibrosis transmembrane regulator comprising the steps of forming a fusion protein comprising a first protein and a polypeptide comprising at least one cystic fibrosis transmembrane regulator domain, employing said fusion protein as an immunogen and collecting antibodies formed in response to said immunogen.
- <sup>132</sup>109. The method of Claim <sup>131</sup>108 wherein said first protein is  $\beta$ -galactosidase.
- <sup>133</sup>110. The method of Claim <sup>131</sup>108 wherein said cystic fibrosis transmembrane regulator domain comprises the region encoded by Exon 13 of the cystic fibrosis transmembrane regulator protein.
- <sup>134</sup>111. The method of Claim <sup>131</sup>108 wherein said cystic fibrosis transmembrane regulator domain comprises a region selected from Exon 1, Exon 10, Exon 24, extracellular loops region of approximately amino acids 139-194 and extracellular loop region of approximately amino acids 881-911
- <sup>135</sup>112. The antibody produced by the method of Claim <sup>131</sup>108.

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